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ADULT AMERICAN DOG TICKS (ACARI: IXODIDAE) AND CANINE-PRODUCED KAIROMONES

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Abstract.—In laboratory bioassays, adult American dog ticks, *Dermacentor variabilis* (Say), of both sexes became akinetic on residues from the hair from the flanks of dogs and from between dog's toes. When confined in petri dishes, female *D. variabilis* became akinetic on or above (on the lid) a canine paw print on a disc of filter paper. These findings help explain the distribution of host-seeking adult *D. variabilis* along trails.

Key Words: kairomone, arrestment, host-finding

The American dog tick, *Dermacentor variabilis* (Say), vector of Rocky Mountain spotted fever in the eastern U.S., is a three host tick. Its larvae and nymphs generally use small mammals as hosts and its adults feed on medium-sized or large mammals (Smith et al. 1946, Sonenshine et al. 1965). Even where common, larvae and nymphs of *D. variabilis* are rarely captured by dragging or flagging, whereas adults are easily picked up on drag cloths or on clothing (Sonenshine et al. 1965).

The propensity of adult *D. variabilis* to wait for hosts along animal trails has been recognized by several authors (Smith et al. 1946, Newhouse 1983, Carroll et al. 1991, Ginsberg 1992). The adaptive value to a tick of waiting along trails that are regularly used by host species instead of waiting at locations that are used little or not used at all by hosts is clear. Although *D. variabilis* can best be categorized as an ambushing type tick, Waladde and Rice (1982), Smith et al. (1946), Sonenshine et al. (1966), Carroll and Nichols (1986) and Carroll et al. (1991) demonstrated the ability of adult *D. variabilis* to disperse several meters. For instance, Carroll et al. (1991) recaptured marked adult *D. variabilis* along trails a few days after they had been released 8m from the trails. Attraction of adult *D. variabilis* of both sexes to host-produced kairomones was inferred by Smith et al. (1946) from a field experiment they conducted on Cape Cod, MA. Stakes and cloths that had been rubbed against the coats of dogs and urinated on by dogs were placed in an area infested with *D. variabilis*. Adult *D. variabilis* were subsequently observed on these treated stakes and cloths, but not on untreated stakes and cloths. It was unclear whether the urine or coat substances attracted and arrested the ticks.

In a laboratory study, Carroll (1999) found that substances rubbed from the dorsal surfaces of dogs' ears elicited an arrestment response (i.e., ceased ambulatory activity in response to a chemical stimulus) in adult lone star ticks, *Amblyomma americanum* (L.), blacklegged ticks, *Ixodes scapularis* Say and *D. variabilis*. The purposes of the present study were to discover whether adult *D. variabilis* responded only to kairomones from particular body regions of

dogs, and whether substances left on the substrate by a dog's feet act as *D. variabilis* arrestment kairomones.

Materials and Methods

Adult *D. variabilis* were collected in Prince George's and Queen Anne's Counties, Maryland, and maintained at 22°C, 95% RH and a photoperiod of 16:8 (L:D) h. Samples of canine coat substances were obtained by rubbing a clean glass rod (15 cm long, 0.5 cm diameter) against the flanks or between the toes of a dog's hind legs. The glass rods were handled only with clean vinyl gloves. The rods were placed in plastic bags according to body region and source animal and refrigerated at 3°C. Samples were taken from 6 different dogs belonging to several breeds (e.g., collie, pekingese, pit bull).

Samples of canine paw prints were obtained by pressing most or all of the plantar surface of a dog's hind paw on a disc of filter paper (Whatman No. 4, 15 cm diameter) marked into quadrants so that the paw only contacted one quadrant. The contacted quadrant was invariably detectable by smudges. Each paper was placed in a separate plastic bag and held at −15 °C until it was used in the bioassay.

Coat substance bioassay.—Ticks were released singly in the middle of small clay islands (2.5 by 1 by 1.3 cm). At one end of each island was a vertical glass rod that had been rubbed against the coat of a dog. At the opposite end of the island was a clean vertical glass rod that had been rubbed between the thumb and forefinger of a vinyl-gloved hand. Each clay island was centered in a plastic petri dish (3.5 cm diameter, 1 cm high) containing water. The petri dish was placed in a second, larger petri dish (10 cm diameter, 1.5 cm high) also containing water. The nested petri dishes containing the clay island were placed in a transparent Plexiglas glove box (65 by 85 by 45 cm) containing water ≈1 cm deep. The water prevented escape of the ticks and maintained high humidity (≈95% R.H.), favorable for the ticks, in the glove box. Tick location was recorded at 1, 18 and 24 h after a tick was released on a clay island. Between bioassays, clay islands were washed with soap and water, and thoroughly rinsed with water. Glass rods were similarly washed and rinsed, and also wiped with a tissue soaked with acetone and a tissue soaked in methanol.

Paw print bioassay.—A piece of filter paper with a paw print in one quadrant was placed in a glass petri dish (10 cm diameter, 1.5 cm high). A tick was placed on the centerpoint of the filter paper and the lid placed on the petri dish. To maintain high humidity in the petri dish, it was placed in a desiccator jar containing water below the rack. The location of the tick was recorded at 1, 18 and 24 h after it was released. Petri dishes were washed with soap and water, and rinsed with tap water between bioassays.

Data from the coat substance bioassays were analyzed by 2 × 2 chi square contingency tables. Tick responses to canine paw prints were analyzed as binomial samples using Fisher's exact test in StatXact (CYTEL) to determine if the response was the same for male and female dogs. Estimates and 95% confidence intervals for the probability of a tick responding to the treated paper (i.e., the tick found akinetic in the quadrant with the print) were calculated for each sex of dog.

Results

Adult *D. variabilis* of both sexes responded positively to substances rubbed from the flanks and from between the toes of dogs (Table 1). At 24 h after their release on clay islands with treated and untreated glass rods, 93.3% of female ticks and 86.7% of male ticks were on glass rods rubbed on dogs' flanks. All 30 female ticks tested and 90% of male ticks tested were on glass rods rubbed between dogs' toes 24 h after the tick's release on the clay island. Ticks responded similarly to substances rubbed from male and female dogs (Table 1).

Table 1. Number of ticks on glass rods rubbed on dogs and on untreated control rods at 24 h after ticks were released on clay island with the 2 glass rods. Thirty ticks of each sex were tested individually against samples from 3 male and 3 female dogs. Treatments and controls may total <30, because some ticks remained on the clay island or fell in the water moat.

		No. Ticks							
		Female Dog		Male Dog		Total of Dogs			
Samples	Sex of Ticks	Treatment	Control	Treatment	Control	Treatment	Control	X^2	*P*
Flank	Female	14	1	14	1	28	2	21.68	<0.0005
	Male	13	1	13	1	26	2	18.49	<0.0005
Toes	Female	15	0	15	0	30	0	28.36	<0.0005
	Male	14	1	13	1	27	2	20.03	<0.0005

In 18 of 25 trials, female *D. variabilis* were on or above (on the petri dish lid) the quadrant of the filter paper that had the paw print of a male dog ($P < 0.0001$) (Table 2). In contrast, only 10 of 25 female *D. variabilis* were on or above the paw prints of female dogs 24 h after the ticks were released in the petri dish. The 40% response of ticks to female paw prints was higher than the expected value of 25%, but it was nevertheless barely within the 95% confidence range of 0.21 to 0.61. Thus the ticks did not respond equally to paw prints from male and female dogs.

Discussion

The distribution of host-seeking adult *D. variabilis* within a given habitat is largely determined by the home ranges of their nymphal hosts, which are frequently small mammals, such as meadow voles, *Microtus pennsylvanicus* (Ord), and white-footed mice, *Peromyscus leucopus* (Rafinesque). Engorged nymphs may move short distances after they drop from their hosts. Because adult *D. variabilis* feed on medium-sized or large mammals, they may be ill-served by remaining at the spot where they metamorphosed from the nymphal stage. Host-seeking *D. variabilis* are capable of moving several meters over a period of days or weeks (Smith et al., 1946; Carroll et al. 1991). However, energy expended on locomotory activities unrelated to host or mate finding or survival (e.g., movement to a microhabitat with a more favorable relative humidity) is wasted. Although, micrometeorological factors (e.g., relative humidity, temperature) determine where host-seeking *D. variabilis* can wait for hosts (Harlan and Foster 1990, Rechav 1979), host-produced chemical cues, such as residues from canine hair and paw prints, further influence where adult *D. variabilis* wait for hosts. The locomotion required in finding a trail or area of host activity depletes a tick's energy reserves, but the expenditure is rewarded by the increased likelihood of contacting a suitable host.

Table 2. Number of adult female *D. variabilis* on quadrant of filter paper with paw print of dog. Five ticks were tested against paw prints of each of 5 dogs (n = 25) for each sex of dog. Ticks on portion of lid of dish directly above quadrant with paw print were considered positive responses.

Female dogs			Male dogs		
No. ticks	%	*P*	No. Ticks	%	*P*
10	40	>0.05	18	72	<0.001

Like a number of ixodids, adult *D. variabilis* are attracted to carbon dioxide sources (Carroll 1988). However, the attraction of *D. variabilis* to carbon dioxide does not adequately explain the occurrence of American dog ticks along trails. Carbon dioxide exhaled by passing animals may start ticks moving toward a trail, but such a source of carbon dioxide is transitory and will probably dissipate before the slow-moving ticks arrive at the trail. On the other hand, host-produced residues on vegetation or sub-

strate may provide a more lasting kairomonal cue. Such kairomonal residues may attract ticks or be contacted by dispersing ticks. The results of the paw print in bioassay showed that the residues from a single paw print can elicit an arrestant response in adult *D. variabilis*. The substrate of a regularly used animal trail may act as a figurative kairomonal magnet to host-seeking *D. variabilis* adults. Once at a trail, a tick is apt to encounter host-produced kairomonal residues on vegetation projecting into the trail. As the bioassays with canine coat substances showed, *D. variabilis* is likely to become akinetic on, above or near the vegetation contacted by hosts, if micrometeorological factors are favorable (Harlan and Foster 1990, Rechav et al. 1979).

Smith et al. (1946) reported the prevalence of adult *D. variabilis* along roads. They suspected that the ticks were attracted to the scent of dogs or people, that some component of automobile emissions might attract the ticks to roadsides, or that roads were barriers to tick dispersal. By marking ticks, they showed *D. variabilis* ticks tend to move to roads and will successfully cross them. Although frequent automobile use would create a gradient of combustion by-products along a road, and possibly have a factitious kairomonal influence on ticks in adjacent woods, the sensitivity of adult *D. variabilis* to canine-produced kairomones supports the host scent explanation for roadside tick distribution. If dogs tended to traverse open areas along roads rather than thickets, *D. variabilis* would also tend to be along the sides of the roads.

In the paw print bioassay, ticks responded with greater frequency to prints made by male dogs than to those made by females. However, because dogs of both sexes use the same trails, ticks drawn to or retained along trails by scents left by male dogs would also be picked up by female dogs. Other suitable host species also run the same trails during some of their activities.

In nature, it is not unusual to find two or more adult *D. variabilis* of the same or both sexes on the same twig or blade of grass. Host-produced kairomones may account for congregations of host-seeking *D. variabilis* at carrion (Kneidel 1984, Carroll and Grasela 1986) but pheromones may also play a role in clustering behavior.

These findings demonstrate the capacity of adult *D. variabilis* to perceive and respond to traces of kairomones rubbed from canine hosts. A paw print alone is sufficient to elicit an arrestant response. Most probably a well-used animal trail is readily recognized by *D. variabilis* adults, which in turn wait there for hosts. Further study is needed to determine the kairomonally active component in canine coat and paw substances, to elucidate any connection between tick responses to kairomones and production of pheromones, and to discover ways in which kairomonally mediated behavior can be manipulated (e.g., traps) to reduce the risk of tick bite and tick-borne diseases.

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